## Remarks

### Summary of the Invention

The present invention provides a fast, flow cytometric assay for detecting antigen-specific T cells by detecting intracellular cytokines following stimulation by contact with a nominal antigen. A critical aspect of the present methods is the combination of selective activation of antigen-specific T cells using a nominal antigen, rather than general activation of T cells in a non-specific manner, with detection using markers (intracellular cytokines) that selectively detect only the desired subpopulation of antigen-activated, antigen-specific T cells.

The present methods provide improved specificity over previously described methods that relied on the expression of cell-surface CD69 to detect antigen-specific T cells following stimulation with a nominal antigen. CD69, expressed only at background levels on resting T cells, increases following T cell activation. However, more T cells express CD69 than proceed to full activation, as measured by expression of intracellular cytokines. In fact, following stimulation with a nominal antigen, the subpopulation that also expresses intracellular cytokines is only a small fraction of the population that expresses CD69. Thus, the present methods enable the selective detection of only those T cells that proceed to full activation following stimulation with a nominal antigen.

### Status Of Claims

Claims 19 - 55 and 61 - 63 are pending.

Claims 19 - 21, 23 - 33, 39 - 40, 43, 45, 47, 49 - 55 and 61 - 63 have been examined.

Claims 22, 34 - 38, 41, 42, 44, 46, and 48 have been withdrawn, to be rejoined upon allowance of a claim generic thereto (37 C.F.R. § 1.146).

Amendments to the Claims

Claim 19 has been amended to describe the invention with greater particularity by incorporating the step recited in dependent Claim 25. Claim 25 has been cancelled in view of the amendment to Claim 19. No new matter has been added.

Supplemental Remarks Regarding the Rejections

Applicant submit the following supplemental remarks to be considered in conjunction with the Supplemental Reply under 37 C.F.R. §1.116, filed April 25, 2002.

Denial of Priority

The present application is a continuation-in-part of U.S. application No. 08/760,447, filed December 6, 1996. Examiner, citing significant differences between the disclosures of the two application, stated that priority is denied. (Paper 28, §5). Applicants respectfully submit that the blanket denial of priority is improper as stated because priority must be determined claim by claim. However, the following remarks regarding the rejections assume, in arguendo, that priority for the claims discussed has been denied. Applicants reserve the right to further traverse the denial of priority if it is restated.

The Rejection under 35 U.S.C. §112, first paragraph

Claims 19-21, 23-33, 40, 43, 45, 47, 49-55, and 61-61 were rejected under 35 U.S.C. §112, first paragraph, on the ground that the specification provides insufficient written description of the generic claim reciting the use of an inhibitor of cytokine secretion (maintained from prior Office action, Paper 24, for reasons stated in Paper 28, §4). Applicants traverse for the reasons set forth below.

In the present methods, an inhibitor of cytokine secretion is used in a manner that is auxiliary to the invention. Its function is to allow intracellular cytokines to accumulate. It is unimportant which inhibitor is used to inhibit cytokine secretion, so long as cytokine secretion is inhibited. The specification provides literal written description of the generic use of an inhibitor of cytokine secretion (see page 5, lines 15-18 and Claim 13 as filed), and exemplifies the use of the preferred inhibitor, Brefeldin A ("BFA").

There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed (see In re Wertheim, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) (''we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims'' (emphasis added)). Applicants believe that this burden has not been met; that persons skilled in the art would recognize in the disclosure a description of the claimed invention.

To address what persons skilled in the art would recognize in the disclosure, Applicants submitted Liabakk et al., 1993, J. Immunol. Methods 163:145-154 (submitted by Fax April 29, 2002), paragraph bridging pages 147-148, paragraph bridging columns 1 and 2 on page 151, and first full paragraph of column 2 on page 152). The reference teaches that, at the time of the invention, the genus of "inhibitors of cytokine secretion" useful in immunoassays was known in the art to comprise two species, monensin and BFA, and that both of these inhibitors could be used for the same purpose[1].

Examiner maintained the rejection in view of both the disclosure in the specification and the knowledge in one of skill in the art, stating:

> However, regardless of what Applicant asserts one of skill in the art would have reasonably believed, no inhibitors of cytokine secretion other than BFA are disclosed in the specification, thus, an insufficient number of inhibitors have been described to support the generic claims.

Paper 28, §4 (emphasis added). Applicants respectfully submit that this rejection is improper because, as discussed more fully, below, the understanding of one of skill in the art must be considered, and the fact that only a single embodiment of a generic method is exemplified does not, by itself, overcome the strong presumption that an adequate written description of the claimed invention is present when the application is filed.

It is well established that in considering the sufficiency of the written description, the specification and claims are reviewed from the standpoint of one of skill in the art at the time of filing (e.g., *Wang Labs. v. Toshiba Corp.*, 993 F.2d 858, 865, 26 USPQ2d

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[1] Applicants note that assuming in arguendo that priority is denied, then Application Note 1 (cited in the rejection under 35 U.S.C. §103) also must be considered as further indication of the state of the art:

> Recently, Jung et al and Picker et al have adapted a method to detect intracellular expression of cytokines after incubation with drugs, such as monensin or Brefeldin A (BFA). This process disrupts intracellular Golgi-mediated transport and allows cytokines to accumulate....

1767, 1774 (Fed. Cir. 1993)). The broadest reasonable interpretation of the claims must also be consistent with the interpretation that those skilled in the art would reach." *In re Cortright*, 49 USPQ2d 1464, 1467 (Fed. Cir. 1999). The art of record shows that one of skill in the art would construe the genus of cytokine secretion inhibitors, in the context of intracellular cytokine assays, to comprise two agents, monensin and BFA, and that these inhibitors of cytokine secretion were known in the art.

The description need only describe in detail that which is new or not conventional (See *Hybritech v. Monoclonal Antibodies*, 802 F.2d at 1384, 231 USPQ at 94 (Fed. Cir. 1986)). The specification describes a known genus; the lack of explicit mention in the specification of monensin is no more than the omission of a detail that is not new. When properly viewed from the standpoint of one of skill in the art, it is clear that such a person of skill in the art would recognize in the disclosure a description of the invention defined by the claims.

It also is well established that there are situations wherein one species adequately supports a genus, particularly where, as in the present case, the genus is used in a manner auxiliary to the invention. For example, in *in re Rasmussen* ("Rasmussen"), the court considered the sufficiency of written description of claims broadened in reissue beyond the scope disclosed in the specification as filed, stating:

> disclosure of a single method of adheringly applying one layer to another was sufficient to support a generic claim to "adheringly applying" because one skilled in the art reading the specification would understand that it is unimportant how the layers are adhered, so long as they are adhered.

*in re Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326–2 (C.C.P.A. 1981). The facts in the present case are even more favorable to Applicants' position than in Rasmussen. In contrast to Rasmussen, there has been no broadening of claim language in the present case, the generic method is described in the specification and in the claims as filed. In accord with Rasmussen, the disclosure of a single method using an inhibitor of cytokine secretion, i.e., the exemplified method using BFA, would be sufficient to support a generic claim reciting "an inhibitor of cytokine secretion" because one skilled in the art reading the specification would understand that it is unimportant which inhibitor is used to inhibit cytokine secretion, so long as cytokine secretion is inhibited. However, the

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Application Note 1, page 1, last paragraph.

specification provides even more written description support, it provides a literal description of the generic invention. In the present case, the disclosure in the specification is more than sufficient to support the claims. Restated, the fact that the specification exemplified only one of the two species that comprise the genus "inhibitors of cytokine secretion" does not suggest that one of skill in the art would not recognize in the disclosure a description of the invention defined by the claims, and certainly does not provide sufficient basis to overcome the strong presumption that an adequate written description of the claimed invention is present when the application is filed.

In summary, the specification describes that the claimed methods are to be carried out using an inhibitor of cytokine secretion. Two, and only two, inhibitors of cytokine secretion were well known in the art at the time of the invention; the specification recommends and exemplifies the use of one of the two. It is clear from the specification that it is unimportant which inhibitor is used to inhibit cytokine secretion, so long as cytokine secretion is inhibited. In view of these facts and the case law discussed above, Applicants submit that the specification fully meets the written description requirement. Applicants respectfully request reconsideration and withdrawal of the rejection of claims under 35 U.S.C. §112, first paragraph, for the reasons discussed above.

The Rejection under 35 U.S.C. §103

Claims 19 – 21, 23 – 33, 39 – 40, 43, 45, 47, 49 – 55, and 61 – 63 were rejected under 35 U.S.C. § 103 as obvious over a combination of three references: Becton Dickinson Application Note 1 ("Application Note 1") in view of Maino et al. and U.S. Patent No. 6,143,299 ("the '299 patent"). The rejection was maintained from prior Office action, Paper 24, for reasons stated in Paper 28, §7.

Applicants previously both traversed the rejection by presenting argument showing the prima facie rejection was improper and by rebutting the prima facie rejection with expert testimony, provided as declarations under 37 C.F.R. §1.132, that, at the time of the invention, one of skill in the art would not have expected the claimed methods to work. However, Examiner maintained the rejection in view of the declaratory evidence rebutting the rejection, stating:

> It is the Examiner's position that the Maino et al. reference provides a sufficient expectation of success. The reference teaches that individual antigen-activated cells are detectable (particularly in light of Applicant's argument that flow cytometry measures only individual events, i.e., individual cells, see Applicant's arguments regarding Priority). The Application Note 1 references teaches additional claimed limitations, i.e., the measure of intracellular cytokines (....) for a product already publicly available. Given the addition of costimulation (to boost activation) and the addition of BFA (to inhibit cytokine secretion and thus, boost signal) in addition to the treaching of the Maino et al. reference that individual antigen-activated cells can indeed be cytometrically assayed, one of skill in the art at the time of the invention would have had a reasonable expectation of success in performing the claimed method with nothing more than routine optimization.

(Paper 28, §7). Applicants maintain, for reasons of record, that the prima facie rejection is improper. Even if, in arguendo, the prima facie rejection is taken as proper, Applicants maintain that the rejection also is improper because it ignores the declaratory evidence provided that specifically contradicts Examiner's position.

The Second Declaration by John D. Altman ("The Altman 2nd Dec"), filed January 28, 2002 (not entered) and again on April 25, 2002 (entered), specifically refutes Examiner's position that it would be obvious to modify the method of Maino et al. by using intracellular cytokines to detect antigen-activate, antigen-specific T-cells instead of using cell-surface CD69. The Altman 2nd Dec, in §§14-18, describes that the expression of CD69 is not coextensive with that of cytokines among T cells exposed to an activating stimulus. In particular, it was known in the art that, after stimulation, the subpopulation of cells that express intracellular cytokines is only a small fraction of the cells that express cell-surface CD69. Altman concludes that:

> Thus, although Maino et al. could perhaps have been read to suggest that T cells activated by contact with nominal antigen in vitro could thereafter be identified by their surface expression of CD69, that is not to say that Maino et al. could be, or would have been, read to suggest that the far smaller subset of T cells that are truly specific for nominal antigen could be identified by flow cytometric measurement of their cytokine expression.

Altman 2nd Dec, §8 (emphasis added). The Altman 2nd Dec provides expert testimony that one of skill in the art at the time of the invention would have expected that the number of antigen-specific T cells that express intracellular cytokines would be too small

to detect using flow cytometric methods, and that this understanding, if anything, would teach away from the expectation that antigen-activated, antigen-specific T cells could be detected by flow cytometrically detecting intracellular cytokines (see §§19-22).

Thus, the Altman 2nd Dec provides expert testimony that (1) Maino et al. would not have been read by one of skill in the art to suggest that the subset of T cells that express cytokines after antigen-specific stimulation could be identified by their intracellular cytokine expression, and (2) that the art of the time had taught that T cells that respond specifically to a nominal antigen would occur at frequencies too low to be detected reliably by flow cytometry. In fact, the unexpected success of Applicants' methods contributed to a fundamental revision in the belief of one of skill in the art as to the frequency of memory T cells (see the Altman 2nd Dec, §22).

The declaratory evidence submitted by Applicants regarding the expectation of one of skill in the art must be considered in an ultimate determination of obviousness. The evidence shows that the rejection is based on a combination that is contrary to accepted wisdom in the art. This evidence is evidence of nonobviousness (see in re Hedges, 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986), and MPEP 2146.X.D.3). For this reason, the rejection is improper and should be withdrawn.

Applicant wish to clarify for the record an inaccuracy in Examiner's statement that Maino et al. teach "that individual antigen-activated cells are detectable", with reference Applicants' earlier arguments regarding priority. Applicants previously pointed out that flow cytometry measures individual events, i.e., individual cells, in response to Examiner's concerns that the priority document describes the methods as detecting T cells, whereas the present application describes the methods as detecting individual T cells. As flow cytometry detects T cells in a population by analyzing the individual cells as they pass by a detector, these two descriptions of the methods are equivalent.

However, although flow cytometry collects data by analyzing individual cells as they flow by a detector, this fact does not, by itself, indicate that resulting data allow the unambiguous detection of a single cell in a large sample of cells. These are separate issues, the first relates to the method by which the data are gathered, the second relates to the resulting data and the statistical problem of distinguishing a small subpopulation of positive events from the "noise" detecting a vastly larger population of negative events.

This holds true whether the data are collected simultaneously, e.g. by measuring overall fluorescence from a labeled subpopulation in a sample of cells, or accumulated cell-by-cell. Applicants' remarks regarding the method by which data are collected by flow cytometry do not speak to the teaching of Maino et al. and do not contradict the declaratory evidence submitted.

In summary, Applicants provided expert testimony that the combination of references upon which the prima facie case is based would have been expected to be inoperable and, thus, the combination would be contrary to accepted wisdom in the art. The expert testimony, which directly refutes the basis for the prima facie rejection, must be considered. Applicants respectfully request reconsideration and withdrawal of the rejection of Claims 19 – 21, 23 – 33, 39 – 40, 43, 45, 47, 49 – 55, and 61 – 63 under 35 U.S.C. § 103 in view of the above remarks.

## Conclusion

Applicants respectfully submit that all rejections have been traversed or rebutted and that the application is in condition for allowance. Applicants respectfully request that the claims that have been withdrawn pursuant to election of species be rejoined and all pending claims be allowed.

Respectfully submitted,

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Date

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**Version With Markings To Show Changes Made**

**Appendix Pursuant to 37 C.F.R. § 1.121(c)(ii)**

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In the Claims:

Please cancel claim 25 without prejudice.

Please amend claim 19 to read as follows:

19. (thrice amended) A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

contacting a sample containing peripheral blood mononuclear cells with a nominal antigen;

adding to said sample an inhibitor of cytokine secretion;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.